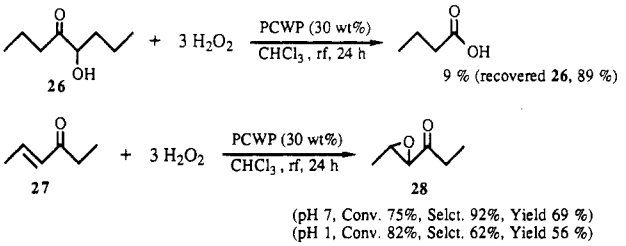
of olefins, was inadequate for the oxidation of alkynes. Table II shows the oxidations of several alkynes by the PCWP-H_2O_2 system.

Although unsymmetrical internal alkynes such as 3-octyne (**6**) afforded all possible isomers, **7** and **9** were formed in preference to **8** and **10**, respectively (run 2). The oxidation of 2,2-dimethyl-3-octyne (**16**) bearing a branched methyl group afforded a complex mixture of products, but a normal epoxy ketone **17** (13%) was obtained together with a rearranged **18** (6%), in which the methyl group migrated to the adjacent carbon atom. A similar 1,2-methyl migration has been observed in the epoxidation of di-*tert*-butylacetylene with MCPBA.[6b] Due to the poor selectivity of the reaction, α,β-unsaturated ketone was difficult to be isolated.

In the oxidation of alkynes involving phenyl group, 1,2-dicarbonyl compounds were formed in fair yields. For instance, diphenyl acetylene (**20**) was selectively oxidized to benzyl (**21**), though the conversion was low (45%). However, the same oxidation in *t*-BuOH resulted exclusively in a cleaved product, benzoic acid (**22**), without formation of **21**. The reaction of alkylaryl acetylene, **23**, took place with difficulty to afford the 1,2-dicarbonyl compound, **24**, in low yield (run 8). Terminal alkynes were cleaved to carboxylic acids (runs 5 and 9).

In order to obtain information about the reaction path, 5-hydroxy-4-octanone (**26**) and an α,β-unsaturated ketone, **27**, were oxidized by the PCWP-H_2O_2 system under the same conditions as the alkynes (eq 2).



26 **27** **28**

(pH 7, Conv. 75%, Selct. 92%, Yield 69 %)
(pH 1, Conv. 82%, Selct. 62%, Yield 56 %)

The reaction of **26** gave a small amount of butyric acid, but most of **26** was recovered unchanged. This finding suggests that the α,β-unsaturated ketone obtained in the oxidation of alkynes is not formed via dehydration of α-hydroxy ketone, but by an alternative route that remains uncertain.

On the other hand, **27** afforded the corresponding epoxide, **28**, in 69% yield (75% conversion and 92% selectivity). Conventionally, such an epoxidation is carried out with the sodium salt of hydrogen peroxide (NaOOH)[17] or the sodium salt of *tert*-butylhydroperoxide (NaOOBu^t),[18] and the reaction is believed to proceed via nucleophilic addition of the hydroperoxide anion at the β-carbon followed by intramolecular displacement of hydroxide ion.[19] However, the present epoxidation of an α,β-unsaturated ketone by the PCWP-H_2O_2 appears to proceed via a different path than that of the alkaline hydrogen peroxide, NaOOH, i.e., it seems likely that the PCWP-catalyzed epoxidation proceeds via an electrophilic attack of peroxo oxygen to the double bond of **27**. This is because the epoxidation of **27** to **28** was effected even under the two-phase system whose aqueous phase was adjusted to pH 1 with sulfric acid (eq 2). Under such a condition the epoxidation with nucleophilic species such as Q^+OOH^- (Q^+: cetylpyridinium ion) may be excluded, since the existence of such a species in the acidic medium is unlikely.[20] Furthermore, the epoxidation of the simple olefin like 1-octene (**29**) to 1,2-epoxyoctane (**30**) by the PCWP-H_2O_2 system, where the reaction involves an electrophilic attack of the peroxo species to the double bond, was completed more rapidly than that of the electron deficient olefin **27** under the same reaction conditions, i.e., **29** was epoxidized to **30** in almost quantitative yield (>98%) within 3 h, while the yield of **28** from **27** was 69% even after 24 h.

It is important to note that the stoichiometric reaction of **27** with PCWP in chloroform gave **28** in 49% yield without any side product. This finding indicates that the peroxo oxygen involving the PCWP possesses a strong electrophilicity which can even epoxidize an electron-deficient olefin such as **27**, and that α,β-unsaturated ketone is a possible precursor of an epoxy ketone.

Although the detailed reaction path is uncertain, the present epoxidation of acetylenes has been of considerable interest from the synthetic and mechanistic points of view as a new class of oxidation by aqueous hydrogen peroxide.

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Chemical Synthesis of Oligoribonucleotides Containing 2-Aminopurine: Substrates for the Investigation of Ribozyme Function

Jennifer A. Doudna,† Jack W. Szostak,† Alexander Rich,‡ and Nassim Usman*,‡

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114

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Summary: The chemical synthesis of a fully protected ribonucleoside phosphoramidite, containing 2-aminopurine as the base component, and its incorporation into short oligoribonucleotides as substrates for an engineered ribozyme from *Tetrahymena* is described.

The ability to chemically synthesize RNA oligomers has made it possible to incorporate modified or unnatural base analogues into short RNA chains. These analogues will be useful for studying details of molecular structure in a number of RNA-protein systems such as tRNA-synthetase interactions, as well as the mechanisms used by various RNA catalysts.[1,2] Analogues of naturally occurring deoxyribonucleotides have recently been prepared for the study of protein-DNA interactions and the development

† Massachusetts General Hospital.
‡ Massachusetts Institute of Technology.

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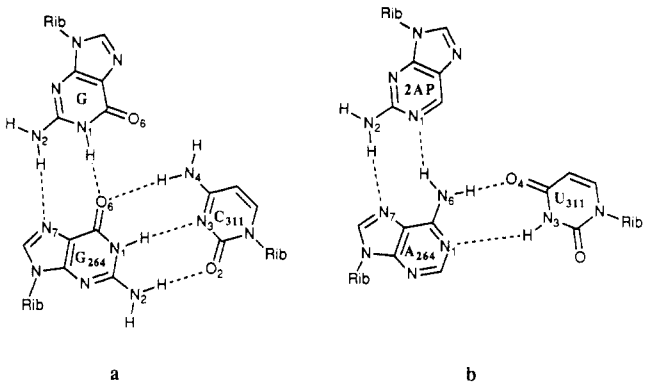

Figure 1. Proposed interaction of *Tetrahymena* ribozymes with their substrates.[5] (a) The G264:C311 base pair in the wild type ribozyme and its interaction with guanosine. (b) The A264:U311 base pair in the altered ribozyme and its interaction with 2-aminopurine ribonucleoside.

of an extended genetic code.[3,4] In this paper we report the synthesis of the protected 2-aminopurine ribonucleoside phosphoramidite and its incorporation into RNA oligomers. We were interested in the 2-aminopurine moiety in particular because of the existence of an altered *Tetrahymena* group I RNA enzyme (ribozyme) which binds to the base of 2-aminopurine ribonucleoside with greater affinity than to its usual substrate, guanosine.[5] Figure 1 illustrates the binding specificity of the two ribozymes. In the altered ribozyme, the wild type G264:C311 base pair (Figure 1a) has been replaced by an A264:U311 base pair (Figure 1b). This new base pair requires a base with one hydrogen bond donor (H–N2) and one hydrogen bond acceptor (N1) in order to form the triple stranded intermediate. This binding specificity requirement is fulfilled by the 2-aminopurine base. Thus, RNA oligomers containing 2-aminopurine may be used to study reactions catalyzed by the altered ribozyme.

The automated solid-phase synthesis of RNA polymers using 2′-O-silylated nucleoside phosphoramidites is an efficient means of generating short RNAs of any desired sequence.[6,7] Hence, we synthesized N^2-benzoyl-5′-*O*-(dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-3′-*O*-((cyanoethyl)diisopropylamino)phosphoramidite of 2-aminopurine ribonucleoside **6**, which could then be used along with the standard phosphoramidites containing the bases Ade, Cyt, Gua, and Ura to make RNA.

The synthesis of **6** involved five steps which are outlined in Figure 2. The starting material, 2-amino-6-thio-9-β-D-ribofuranosylpurine (**1**) (obtained from Aldrich Chemical Company), was quantitatively reduced to 2-amino-9-β-D-ribofuranosylpurine (**2**) using an aqueous suspension of Raney Ni.[8] In the second step, the amino group of **2** was protected using the transient silylation method.[9,10] This reaction, following the extraction of the pyridine/water

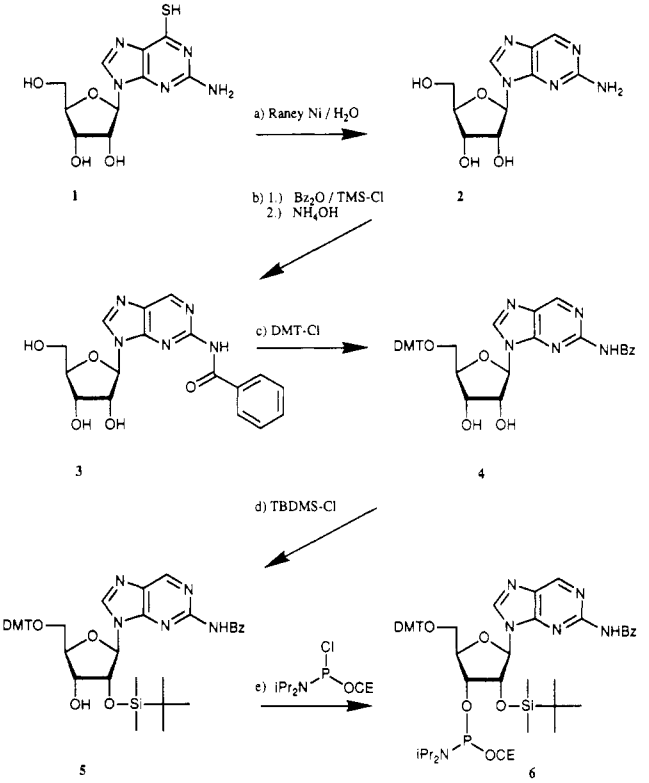

Figure 2. Synthetic scheme for 2-aminopurine. (a) Raney Ni (cat.), water, 100 °C (reflux), 2 h. (b) Chlorotrimethylsilane (7.5 equiv), pyridine, 25 °C, 15 min; benzoyl chloride (3 equiv), 2 h. (c) Dimethoxytrityl (DMT) chloride (1.2 equiv), (dimethylamino)pyridine (cat.), triethylamine (1.4 equiv), pyridine, 0 °C, 12 h. (d) *tert*-Butylchlorodimethylsilane (1.2 equiv), imidazole (4 equiv), pyridine, 0 °C, 5 h. (e) (*N*,*N*-Diisopropylamino)(cyanoethyl)phosphonamidic chloride (1.5 equiv), diisopropylethylamine (5 equiv), *N*-methylimidazole (cat.), tetrahydrofuran, 25 °C, 1 h.

phase with methylene chloride and evaporation of the pyridine/water layer in vacuo, yielded N^2-benzoyl-2-aminopurine ribonucleoside **3** in 90% yield. The 5′-hydroxyl of this compound was protected using dimethoxytrityl chloride.[11] The resulting tritylated compound **4** was purified by silica gel chromatography.

The purified product **4** was next treated with *tert*-butylchlorodimethylsilane to protect the 2′-hydroxyl on the ribose ring.[12-14] The desired 2′-*O*-silyl derivative **5** was obtained in 45% yield following silica gel chromatography.[15] In the final step of the synthesis, N^2-benzoyl-5′-*O*-(dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-2-aminopurine ribonucleoside **5** was phosphitylated using (*N*,*N*-diisopropylamino)(cyanoethyl)phosphonamidic chloride.[6] The product **6** was purified by silica gel chromatography in 98% yield. All intermediates were characterized by thin-layer chromatography, UV spectrophotometry, and proton NMR. The final product **6** was further characterized by ^{31}P NMR.[16]

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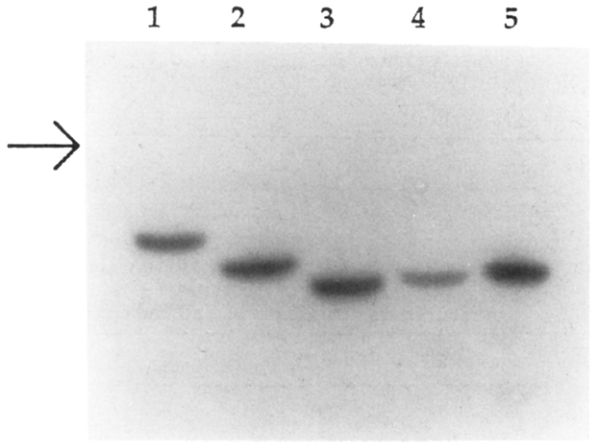

Figure 3. Autoradiogram of 20% polyacrylamide/7 M urea gel containing RNA tetramers 5′-end-labeled with ^{32}P. Following deprotection, the tetramers were desalted on Sephadex G10 columns and 1 OD unit of each was loaded onto a Dionex NucleoPak NA100 anion exchange column. RNA was eluted with a binary buffer system consisting of buffer A (25 mM ammonium acetate, pH 5.2) and buffer B (1 M ammonium acetate, pH 5.2). A gradient of 100% A, 0% B to 50% A, 50% B was used to elute the RNA tetramers. Peak fractions were lyophilized and resuspended in deionized water. For each sample, 200 pmol were phosphorylated using polynucleotide kinase and γ-^{32}P ATP.[23] Lane 1, pGAAG; lane 2, pGAAC; lane 3, p2AP-AAC; lane 4, p2AP-AAG; lane 5, p2AP-AAU. Arrow indicates the position of bromophenol blue dye in the gel.

The protected 2-aminopurine phosphoramidite was incorporated into RNA oligomers using a Milligen/Biosearch Cyclone DNA synthesizer. The coupling program (for 1-μmol scale synthesis) was modified to duplicate the procedure developed for RNA synthesis essentially as described previously.[6,7] Protected ribonucleoside phosphoramidites with the general structure **6** except with N^6-benzoyladenine, N^4-benzoylcytosine, N^2-benzoylguanine, and uracil as the base component were obtained from Milligen/Biosearch Inc., Novato, CA. Each was at least 97% pure as determined by ^{31}P NMR spectra of 50-mg samples.

We made several different tetramers in which 2-aminopurine was the 5′-base. Overall coupling yields were determined to be 95–97% according to the A_{504} of the released trityl cations. The tetramers were deprotected as described.[6] Following treatment with tetra-*n*-butylammonium fluoride and quenching with ammonium acetate, the crude tetramers were desalted on Sephadex G10 columns. Yields of crude tetramers ranged from 20–24 OD units.

Purification of the tetramers was necessary in order to remove incompletely deprotected molecules and shorter sequences. Three different methods were tested: denaturing 20% polyacrylamide gel electrophoresis, BND cellulose column chromatography, and HPLC chromatography. We found gel purification to be the least satisfactory method, since heterogeneous material migrated at essentially one position in the gel matrix.[17] BND cellulose was useful for removing incompletely deprotected molecules but not shorter products. HPLC chromatography of crude desalted tetramers was performed using a Dionex NucleoPak NA100 anion-exchange column and gave very good results. Tetramers purified in this way were free of detectable contaminants (protected nucleotides, 5′–2′ linked dimers, and shorter oligomers) as determined by enzymatic degradation and two-dimensional thin-layer chromatography.[18–20] Figure 3 shows an autoradiogram of a 20% polyacrylamide gel containing 5′-end-labeled purified tetramers.

Preliminary experiments indicate that these tetramers are used efficiently and specifically by the altered *Tetrahymena* ribozyme in ligation reactions[21] similar to those described previously.[22] We hope ultimately to use the 2-aminopurine containing oligomers as substrates for a self-replicating RNA based on the modified ribozyme. Since the methodology for making ribonucleoside phosphoramidites is fairly straightforward, we expect that the use of base analogues to study other questions of RNA structure and function will become increasingly common.

Acknowledgment. We thank Milligen/Biosearch for providing the Cyclone DNA synthesizer and the standard ribonucleoside phosphoramidites, Mr. Adam Foley for obtaining NMR spectra, Mr. Steven Scaringe for technical assistance, and Dr. Loren Williams for critical reading of this manuscript. This research was supported by funds from the National Institutes of Health, The National Science Foundation, the Office of Naval Research, the National Aeronautics & Space Administration, and Hoechst A.G. N.U. was a recipient of an NIH Fogarty International Research Fellowship, 1988–89.

(16) Two diastereomers, a and b, respectively, in $CDCl_3$. ^{31}P NMR: δ 152.234, 149.361 ppm. ^1H NMR (ppm) 9.18, 9.15 (s, 1, H_8); 8.14, 8.11 (s, 1, H_6); 5.96, 5.77 (d, $J_{1'-2'}$ = 7.8 Hz, 1, $H_{1'}$), 5.30, 5.40 (dd, $J_{2'-3'}$ = 4.9 Hz, 1, $H_{2'}$); 4.32, 4.31 (m, $J_{3'-4'} \approx$ 1 Hz, 1, $H_{3'}$); 4.25, 4.36 (m, $J_{4'-5'} \approx$ 7 Hz, $J_{4'-5''}$ = 3.0 Hz, 1, $H_{4'}$); 3.97, 3.88 (m, $J_{P-H} \approx$ 7.8 Hz, 2, $POCH_2CH_2CN$); 3.65, 3.64, 3.63, 3.62 (s, 3, 2 OCH_3 (DMT)); 3.48, 3.48 (dd, $J_{5'-5''}$ = 10.6 Hz, 1, $H_{5'}$); 3.09, 3.05 (dd, 1, $H_{5''}$); 2.67 (t, J_{H-H} = 6.6 Hz, 2, $POCH_2CH_2CN$); 0.71, 0.70 (s, 9, Si-*t*-Bu); −0.07, −0.08, −0.32, −0.34 (s, 3, 2 $Si(CH_3)$). TLC: R_f = 0.34 $Et_2O/CHCl_3$ (1/1) + 1% MeOH. UV (95% EtOH): λ_{max} = 272 nm, λ_{min} = 257 nm. N.B.: The pure 3′-TBDMS, 2′-phosphoramidite was also prepared for comparison. ^{31}P NMR: δ 151.475, 150.378 ppm.

(17) This difficulty arises from the aberrant gel mobility of short oligonucleotides which is the result of a large difference in charge/mass ratio, z/m, among the short oligonucleotides. e.g. z/m = 0.5, 0.66, 0.75, 0.8, and 0.83 for dimers, trimers, tetramers, pentamers, and hexamers respectively using z = 1 for each phosphate residue and m = 1 for each nucleoside residue. As the z/m ratio approaches 1 the gel mobility of an oligonucleotide is based solely on its mass (i.e. "normal" mobility, wherein the smaller the fragment is, the faster its mobility). However, in the case of the shorter oligomers both charge and mass play a role in mobility, which results in a reversal of the "normal" mobility. This has been verified by 5′-end-labeling small oligomers, which results in a z/m = 1 for all species. This restores the "normal" mobility (unpublished data).

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